SARS-CoV-2 Bioassays

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Antibodies produced from viral vaccines have been reported to provide protection against viral infection through two major mechanisms.

1. Neutralizing antibodies prevent new rounds of infection by preventing viral binding and entry of host cells.

2. Antibodies can mediate ADCC or ADCP that results in the clearance of infected cells by immune effector cells.
Dual MoA for Many SARS-CoV-2 Antibodies
Outline

Two SARS-CoV-2 Bioassays are developed to address the two major Mechanisms of Actions for SARS-CoV-2 antibodies.

1. SARS-CoV-2 HiBiT-PsVLP Assay for neutralization activity

2. ADCC/ADCP Reporter Bioassays for antibody Fc effector function
SARS-CoV-2 HiBiT-PsVLP Assay to Measure the Blocking Activity for Small Molecule Inhibitors and Neutralizing Antibodies
Pseudotyped Virus-Like Particles (PsVLPs)

Virus-like particles (VLPs): non-replicating nanostructures comprised of viral structural proteins and a lipid envelope; VLPs are non-infectious because they contain no viral genetic material.

- HIV-1 Gag polyprotein can self-assemble into VLPs in the absence of other viral factors.
- Gag VLPs can be pseudotyped (PsVLPs) with heterologous viral envelope proteins to permit cellular entry.

Goal: Generate a safe, rapid, and quantitative assay to monitor SARS-CoV-2 entry using HiBiT-tagged PsVLPs.
**SARS-CoV-2 HiBiT-PsVLP Assay Design**

**Assay Design:**

1. HiBiT-tagged VLPs pseudotyped with SARS-CoV-2 Spike protein are added to SARS-CoV-2 Target Cells
   - HiBiT is packaged inside the PsVLPs

2. In the absence of inhibitors or neutralizing antibodies (nAbs), SARS-CoV-2 HiBiT-PsVLPs bind to target cells via Spike/ACE2 interaction and undergo membrane fusion mediated by cellular proteases. HiBiT is released into target cells and binds to LgBiT to generate a luminescent signal in the presence of substrate.

3. In the presence of inhibitors or nAbs of SARS-CoV-2 entry, the entry/fusion processes of PsVLPs are blocked, thereby preventing HiBiT release. No luminescent signal is produced.
SARS-CoV-2 HiBiT-PsVLP Assay

Assay Workflow

1. **Prepare HiBiT-PsVLPs ± Blocking Abs**
2. **Plate SARS-CoV-2 Target Cells**
   - Pre-treat with cell-targeted inhibitor(s)
   - Add live cell detection Reagent
3. **Add PsVLPs to target cells**
4. **Measure Luminescence**
5. **1 hour**
6. **3 hours**
7. **15 mins**

**Simple, fast, real-time, quantitative!**
Measure the Activity for Neutralizing Antibodies and Small Molecule Inhibitors

SARS-CoV-2 PsVLP Entry

- is inhibited by anti-spike neutralization antibody
- Is inhibited by protease inhibitors

- **E-64d** = Cathepsin inhibitor – targets the endosomal entry pathway
- **Camostat mesylate** = TMPRSS2 inhibitor – targets the cell surface entry pathway
Evaluating the Neutralization Activities against SARS-CoV-2 Spike Variants

- SA variant is resistant to neutralization by the anti-Spike-RBD Ab tested
- TMPRSS2 inhibitor efficiently reduced the entry for all SARS-CoV-2 HiBiT-PsVLP variants

Three SARS-CoV-2 PsVLP carrying spike proteins with D614G mutation, major mutations reported for UK variant or South African variant were produced and tested in the assay.
Summary of SARS-CoV-2 HiBiT-PsVLP Assay

1. Increased biosafety
   - HiBiT-PsVLPs are non-replicating
   - No viral genome present

2. Simple and rapid
   - No gene expression steps required for assay readout
   - Monitor viral entry in live cells in real time

3. PsVLPs and Target Cells offered in Thaw-and-Use format
   - No need to generate live virus or pseudovirus
   - No need to culture cells

4. Quantitative assay readout
ADCC/ADCP Reporter Bioassays to Measure the Fc Function for SARS-CoV-2 Antibodies
Reporter Bioassays to Measure Fc Function (ADCC, ADCP) for SARS-CoV-2 Antibodies

1) Anti-spike antibody binds to S protein on target cells and FcγR* on FcγR Reporter Bioassay Effector Cells simultaneously.

2) It leads to the activation of FcγR receptor and luciferase activation in the reporter effector Cells.

*ADCC Reporter Bioassay: FcγRIIIa
*ADCP THP-1 Reporter Bioassay: FcγRIIa, FcγRI
## Commercial SARS-CoV-2 Antibodies Evaluated

<table>
<thead>
<tr>
<th>Test Ab</th>
<th>anti-SARS-CoV-2 S Ab</th>
<th>vendor</th>
<th>CAT#</th>
<th>IgG isotype</th>
<th>Specificity</th>
<th>Neutralization Activity</th>
<th>Reference</th>
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<td>Clone 105-9</td>
<td>Biolegend</td>
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<td>Biolegend</td>
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<td>S2</td>
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</table>

References:
ADCC Reporter Activities for anti-SARS-CoV-2 Spike Antibodies

- Seven anti-SARS-CoV-2 S antibodies were tested in ADCC Reporter Bioassay using SARS-CoV-2 S CHO-K1 target cells.

- Four antibodies, Ab1, 3, 6 and 7 showed positive ADCC activity.

<table>
<thead>
<tr>
<th></th>
<th>Ab 1</th>
<th>Ab 2</th>
<th>Ab 3</th>
<th>Ab 4</th>
<th>Ab 5</th>
<th>Ab 6</th>
<th>Ab 7</th>
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<td>EC50, mg/ml</td>
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<td>4.3</td>
<td>1.4</td>
<td>1.1</td>
<td>2.7</td>
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ADCP Reporter Activities for anti-SARS-CoV-2 Spike Antibodies

- Four anti-SARS-CoV-2 S Antibodies, Ab1, 3, 6 and 7 were tested in ADCP THP-1 Reporter Bioassay using SARS-CoV-2 S CHO-K1 target cells.

- All four antibodies tested showed strong ADCP reporter activity.

<table>
<thead>
<tr>
<th></th>
<th>Ab 1</th>
<th>Ab 3</th>
<th>Ab 6</th>
<th>Ab 7</th>
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<td>EC50, mg/ml</td>
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Summary of SARS-CoV-2 ADCC/ADCP Assays

• Can quantitatively measure ADCC and ADCP activity for SARS-CoV-2 Abs.

• Can measure Ab Fc function in the presence of human serum (data not shown), indicating the potential use for patient's samples after vaccine administration.
Thank You

https://www.promega.com/resources/events/global/biologics-2020-resources/

https://www.promega.com/applications/biologics-drug-discovery/